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HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEW

Genetics of Leptin and Obesity: A HuGE Review

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Leptin is an important regulator of the mass of adipose tissue and of body weight; it operates by inhibiting food intake and stimulating energy expenditure. Some polymorphic genes involved in the regulation of leptin—the leptin gene (*LEP* A19G), the leptin receptor gene (*LEPR* Q223R, K109R, and K656N), and the peroxisome proliferator-activated receptor-gamma gene (*PPARG* P12A and C161T)—have been investigated as possible factors associated with obesity. Allelic frequencies of these polymorphisms show ethnic variation. The authors performed a meta-analysis of the available data on the association between these polymorphisms and obesity based on case-control studies. Odds ratios and 95% confidence intervals for obesity associated with leptin polymorphisms were calculated by using both fixed- and random-effects models. Results suggest no evidence of association between the genes under study and obesity. The lack of association could be due to the complex pathogenesis of obesity, which involves a number of genetic and environmental factors. Large studies including testing of multiple genes in both obese and lean subjects, with epidemiologic data on dietary habits in different ethnic groups, are necessary to better understand the role of leptin in regulating weight in human populations.

epidemiology; genetics; *LEP*; *LEPR*; leptin; meta-analysis; obesity; *PPARG*

Abbreviations: BMI, body mass index; CI, confidence interval; *LEP*, leptin gene; *LEPR*, leptin receptor gene; *PPARG*, peroxisome proliferator-activated receptor-gamma gene.

Editor's note: *This paper is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).*

Leptin is a 167-amino acid protein produced by the leptin gene (*LEP*), whose name is derived from the Greek word ''leptos,'' which means ''thin.'' Leptin, an important signal in the regulation of adipose-tissue mass and body weight, operates by inhibiting food intake and stimulating energy expenditure. Defects in leptin production cause severe obesity (http://www.ncbi.nlm.nih.gov/entrez/). Leptin is also known to play a role in different parts of the body, such as the male and female reproductive organs, the mammary gland, bone mineral density, the immune system, the gut, the kidney, and the lung (1).

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Many of the genes involved in the regulation of leptin are polymorphic. This paper reviews polymorphisms in three genes—*LEP*, the leptin receptor gene (*LEPR*), and the peroxisome proliferator-activated receptor-gamma gene (*PPARG*)—and their association with obesity.

BIOLOGY OF LEPTIN

The structure of leptin consists of a complex of four helices, similar to that of cytokines. Leptin is produced by the white adipose tissue, the most frequent form of adipose tissue in mammals. The white adipose tissue provides a long-term fuel reserve that can be mobilized during food deprivation with the release of fatty acids for oxidation in other organs; it also provides thermal insulation and has a mechanical role for vital organs (2). Leptin is produced in many sites in addition to white adipose tissue, but the amount of body fat is the main determinant of the circulating levels of this hormone. After it is produced, leptin is secreted into the bloodstream, where it circulates attached to proteins, and is transported to the brain, where it stimulates or inhibits release of several neurotransmitters. It down-regulates some orexigenic neuropeptides, such as the neuropeptide Y, melanin-concentrating hormone, orexins, and agouti-related peptide. Leptin up-regulates anorexigenic neuropeptides such as alpha-melanocyte-stimulating hormone, which acts on the melanocortin-4 receptor, cocaine and amphetamine-regulated transcripts, and corticotropin-releasing-hormone (3). Leptin may also directly affect the metabolism and function of peripheral tissues such as the adipocytes, skeletal muscle, ovary, adrenal cortex, and pancreatic beta cells (4, 5).

Leptin is expressed in the adipocytes: both its expression and its secretion are highly correlated with body fat and adipocyte size (4). However, the study of serum leptin levels in relation to several measures of adiposity demonstrates that obesity is not characterized by leptin deficiency but rather by hyperleptinemia; in fact, leptin levels have been found to be elevated in obese patients (6, 7). The inability of such elevated leptin levels to alter the obese state of these persons may be related to ''leptin resistance,'' an inability of leptin to enter the cerebral spinal fluid to reach the hypothalamus regions that regulate appetite, or it may simply reflect the large amount of fat tissue in these persons (4). Serum leptin levels follow a circadian rhythm, which seems to be predominantly related to an increase in insulin and cortisol rhythm. These two hormones may be among the major regulators of leptin production by the adipose tissue. Compared with males, females have higher leptin levels if leptin levels are expressed as a percentage of body adiposity (8). This difference may be related to the different distribution of adipose tissue in the two genders as well as to hormonal differences. Leptin levels have been shown to differ with medical conditions involving the endocrine system. For example, a comparison between diabetic and nondiabetic subjects shows that leptin levels are lower in the diabetic population (9).

The biologic activities of leptin on target tissues are carried out through selective binding to a specific receptor, *LEPR*. This gene is found in many tissues in several alternatively spliced forms.

GENES

LEP

The *LEP* gene encodes for leptin. It has been localized in humans on the 7alpha31.3 chromosome and consists of three exons separated by two introns (10).

It has been suggested that the ''obese'' promoter is a natural target of CCAAT/enhancer-binding protein-alpha, a transcription factor implicated in the development and metabolic regulation of adipocytes (11). Overexpression of the human *LEP* gene has been found in both subcutaneous and omental adipose tissue of massively obese persons (12).

LEP gene expression may be influenced by modulation of CCAAT/enhancer-binding protein-alpha levels or activity (13). Knowledge of the sequence elements and factors regulating *LEP* gene expression as well as the precise definition of the structure of the gene could be relevant when conducting further studies on mutations in this gene that may predispose to certain forms of obesity.

LEPR

LEPR maps in humans to the 1p31 chromosome and has at least five isoforms. The extracellular and transmembrane domains are identical between the short and the long isoforms; differences are due to changes in the length of the cytoplasmic domain. The long form (*LEPR1*) has 302 cytoplasmic residues compared with the short form (*LEPRs*), whose cytoplasmic residues range from 32 to 40 amino acids in length. Another form of the leptin receptor, the soluble form (*LEPRe*), is supposed to contain nonintracellular motifs or transmembrane residues, thus consisting entirely of the extracellular domain of the receptor (5).

Isoforms of the receptor have been identified in multiple tissues, such as the pituitary gland, male and female reproductive organs, mammary gland, immune system, gut, kidney, and lung (1). Studies performed on mice showed that the long form, thought to be the most important for transmitting the leptin signal to the cells, is located predominantly in the hypothalamus and not in most other tissues (14), whereas the short forms are expressed throughout the body, especially in the kidney, lungs, and choroid plexus (15).

The structure of the leptin receptor is similar to that of the helical cytokine receptor (class I). Leptin receptors form homodimers, which are capable of activating Janus kinases. The Janus kinase is then able to start activators of the transcription family. Leptin signaling via the Janus kinases–start activators of transcription system is associated largely with the *LEPR1* form (16).

PPARG

The peroxisome proliferator-activated receptor genes (*PPARs*) are members of the nuclear hormone receptor subfamily of transcription factors that is expressed predominantly in the adipose tissue and the immune system. Such receptors control adipocyte differentiation and regulate glucose and lipid homeostasis (http://www.ncbi.nlm.nih.gov/entrez/). *PPARs* form heterodimers with retinoid X receptors (*RXRs*), which regulate the transcription of various

genes implicated in the control of lipid metabolism, insulin synthesis, carcinogenesis, and inflammation (17, 18). There are three known subtypes of *PPARs*: *PPARA*, *PPARD*, and *PPARG*. *PPARG* is located on the 3p25 chromosome and is thought to be involved in adipocyte differentiation (19).

PPARG1 and *PPARG2* are members of the family of orphan nuclear receptors that function as transactivators of fat-specific genes and thus are dominant activators of fat cell differentiation. The *PPARG* gene contains nine exons and spans more than 100 kb. *PPARG1* is encoded by eight exons and *PPARG2* by seven exons (20). Human *PPARG* is expressed at high levels in adipocytes and at a much lower level in the bone marrow, spleen, testis, brain, skeletal muscle, and liver (21).

GENES VARIANTS

LEP

A mutation in the mouse *LEP* gene was first described in 1950 (22): the ob/ob obese mouse shows a nonsense mutation in codon 105 of the original mouse strain (23) resulting in the absence of leptin production. This mutation causes obesity, hyperphagia, hypothermia, extreme insulin resistance, and infertility. Administration of leptin restores the normal condition. The homologous *LEP* mutation in humans has not been detected (24). The structure of the *LEP* gene is preserved in all mammalians: human leptin and mouse leptin share 84 percent sequence identity. In humans, a mutation in the *LEP* gene was reported in two children with the same consanguineous pedigree (25). The homozygous frameshift mutation was the result of deletion of a single guanine nucleotide in codon 133. These children produced a very small quantity of leptin and presented with early-onset obesity and hyperphagia but normal body temperatures and normal plasma cortisol and glucose concentrations. Other variants of the human *LEP* gene have been reported: a rare mutation at codon F17L, a rare mutation at codon V110M (26), and a polymorphism C(-188)A in the promoter region of the *LEP* gene (27) have been found. Different microsatellite markers flanking the *LEP* gene have been also identified, but the possible linkages with obesity are inconsistent. Other studies (28–31) reported a polymorphism in the promoter untranslated exon 1 of the *LEP* gene (A19G).

LEPR

The *LEPR* gene maps to chromosome 4 of the mouse in regions that contain the db/db and Zucker fa/fa mutations. These mutations cause severe obesity in mouse, not reversible by administration of leptin. Human *LEPR* and the mouse gene share 78 percent homology. Several variants commonly occur, which cause two nonconservative changes: glutamine to arginine at codon 223 (CAG to CGG) in exon 6 (Q223R) and lysine to asparagine at codon 656 (AAG to AAC) in exon 14 (K656N); a conservative change: lysine to arginine at codon 109 (AAG to AGG) in exon 4 (K109R); a silent TC change at codon 343; and a silent GA transition at codon 1019. One study found that the leptin receptor is expressed in the hypothalamus of human subjects (32). It has been suggested that leptin responsiveness to energy restriction is affected by the functionality of the leptin receptor. No effect of the Q223R, K109R, and K656N polymorphisms of the *LEPR* gene was observed on the acute decline in leptin after energy restriction (33).

PPARG

A screening of the *PPARG* gene for sequence variants has allowed identification of several genetic variants. P115Q is a very rare gain-of-function mutation associated with obesity (34); V290M and P467L are two loss-of-function mutations reported in three persons with severe insulin resistance but normal body weight (35). Two common polymorphisms are 1) a CG substitution in exon B, resulting in conversion of proline to alanine at residue 12 of the *PPARG* protein (P12A), which may modify susceptibility to type II diabetes mellitus and obesity (36); and 2) a synonymous CT substitution at nucleotide position 161 in exon 6 (C161T).

The Pro12Ala polymorphism has been shown to prevent insulin resistance and obesity induced by a high-fat diet (37). Expression of the P12A polymorphism is not significantly different in obese subjects carrying one or the other variant of the allele (38).

META-ANALYSIS

To examine the frequencies of the polymorphisms in the general population and the association between the various genes involved in regulation of leptin and obesity, we performed a meta-analysis of published studies, following established guidelines (39–41). To investigate the presence of publication bias, funnel plots were created, and their asymmetry was tested statistically: Begg's adjusted rank correlation (42) and Egger's regression asymmetry of the Ln(odds ratio) over the standard error (43) were calculated.

To estimate the heterogeneity of the allele frequencies and of the odds ratios, the Cochran Q-test was performed (44). For the meta-allele frequencies of each specific allele and the meta–odds ratios, a fixed-effects model was used if the assumption of homogeneity among studies could be accepted, while a random-effects model was used when heterogeneity across studies was statistically observed (45).

Population frequencies

A MEDLINE search was performed up to November 2004 by using different combinations of the keywords "leptin," "gene," "polymorphisms," "leptin gene receptor," "*PPARG* polymorphism," "obesity," and "BMI." We also searched for specific polymorphisms of the *LEP*, *LEPR* (Q223R, K109R, K656N), and *PPARG* (C161T, P12A) genes. The search was restricted to articles published in English on human subjects, although we checked the number of articles published in other languages and found that such articles represented 7 percent of the whole relevant literature. The computer search was supplemented by consulting the bibliographies of the articles found through the MEDLINE search. Case-control or cohort, genotype-based studies that

TABLE 1. Studies that included data on the A19G polymorphism of the *LEP gene in healthy subjects**

Authors (reference no.), year	Country	Population	Source	No. of subjects	Gender	*19G* allele frequency	95% CI*
Karvonen et al. (29), 1998	Finland	Caucasian	Weight reduction study, population	206	Mix	0.67	0.61, 0.74
Hager et al. (30), 1998	France	Caucasian	Hospital	108	Mix	0.36	0.27, 0.45
Lucantoni et al. (31), 2000	Italy	Caucasian	Hospital	61	Mix	0.38	0.26, 0.50
Overall		Caucasian		375		0.46	0.29, 0.73
						$Q^* = 27.88$; $p < 0.0001$	

* *LEP*, leptin gene; CI, confidence interval; *Q*, Cochran *Q*-test for heterogeneity.

reported allele frequency for polymorphisms of *LEP*, *LEPR*, and *PPARG* genes in healthy subjects (both lean and obese subjects and excluding clinically diagnosed diabetes) were selected, whereas studies including only diabetic or non-healthy subjects and studies on families or twins were excluded. Allelic frequency of the different polymorphisms for each of the above-mentioned genes were evaluated. For each study and for each gene included in the present review, allele frequencies and 95 percent confidence intervals for healthy subjects were calculated. Pooled frequencies were calculated on the whole sample and according to ethnicity.

Associations

Case-control studies reporting allele frequency for lean (controls) and obese (cases) subjects were included in the meta-analysis. For each study and for each gene, we calculated crude odds ratios and 95 percent confidence intervals as a measure of the association between a gene polymorphism and obesity. The overall odds ratios and 95 percent confidence intervals for obesity associated with leptin polymorphisms were calculated after pooling the data from the single studies. Forests plots were used to convey the results of the meta-analysis.

POPULATION FREQUENCIES

LEP A19G

Three studies conducted in Europe (18–20) reported allele frequency in healthy subjects. Table 1 describes the studies. The allelic frequency of *19G* for the Caucasian population (375 subjects) was 0.46 (95 percent confidence interval (CI): 0.29, 0.73), with comparable values in France and Italy and higher values in Finland. The Cochran *Q*-test indicated heterogeneity among studies ($Q = 27.88$; $p < 0.0001$).

LEPR Q223R

Frequencies of this polymorphism were reported in 18 articles (46–63), and the studies are described in table 2. Five of the studies were conducted in the United States (46, 59–62), three in Asia (56–58), eight in Europe (47–54), and two in Oceania (55, 63). The allelic frequency varied significantly across different countries and ethnic groups ($p < 0.0001$). In particular, the frequency of the *223R* allele for Asians was significantly higher than for other ethnicities. The Cochran *Q*-test showed heterogeneity among only those studies performed among Caucasians ($Q = 42.70$; $p < 0.0001$).

LEPR K109R

Eleven articles (table 2) were reviewed for this polymorphism (47, 49–54, 56, 58, 60, 61); two studies were conducted in the United States (60, 61), two in Asia (56, 58), and seven in Europe (47, 49–54). The frequency of the *109R* allele was significantly different among ethnic groups, being higher in Asians than in other populations (table 2). A significant asymmetry of the funnel plots of the studies performed among Caucasians ($p_{\text{Egger}} = 0.03$) indicates the possible presence of publication bias.

LEPR K656N

Ten articles (46, 47, 49–54, 56, 61) were included in the present analysis (table 2); two studies were conducted in the United States (46, 61), one in Asia (56), and seven in Europe (47, 49–54). The frequency of the *656N* allele showed differences among ethnic groups; the polymorphic allele was more frequent in Caucasians than in Asians (table 2).

PPARG C161T

Six articles, described in table 3, were reviewed for this polymorphism (64–69); one study was conducted in the United States on Pima Indians (68), one in Asia (69), three in Europe (64–66), and one in Oceania (67). The *161T* allele showed similar frequencies in Caucasians and Asians, while the frequency was higher in Pima Indians (table 3).

PPARG P12A

The frequency of this polymorphism in healthy populations was reported in 26 articles (64, 68, 70–93). Table 4 describes the studies. Six were conducted in the United States

TABLE 2. Studies that included data on *LEPR gene polymorphisms in healthy subjects**

Authors (reference no.), year	Country	Population	Source	No. of subjects	Gender	*223R* allele frequency	95% CI*	*109R* allele frequency	95% CI	*656N* allele frequency	95% CI
Silver et al. (46), 1997	United States	Caucasian	University weight center, Baltimore Longitudinal Study on Aging	388	Mix	0.45	0.40, 0.50			0.18	0.14, 0.22
Gotoda et al. (47), 1997	England	Caucasian	Population-based epidemiologic study	322	Male	0.44	0.38, 0.49	0.27	0.22, 0.31	0.17	0.10, 0.24
Quinton et al. (48), 2001	England	Caucasian	Population	88	Female	0.41	0.31, 0.52				
Mammès et al. (49), 2001	France	Caucasian	Hospital, unrelated family study	566	Mix	0.44	0.39, 0.48	0.25	0.21, 0.28	0.18	0.15, 0.22
Wauters et al. (50), 2001	Belgium	Caucasian	Obesity clinic	280	Female	0.48	0.42, 0.54	0.28	0.22, 0.33	0.18	0.14, 0.23
van Rossum et al. (51), 2002	The Netherlands	Caucasian	Cohorts	582	Mix	0.44	0.40, 0.48	0.27	0.24, 0.31	0.19	0.16, 0.22
Rosmond et al. (52), 2000	Sweden	Caucasian	Cohort study	269	Male	0.50	0.44, 0.56	0.24	0.19, 0.29	0.15	0.11, 0.19
Echwald et al. (53), 1997	Denmark	Caucasian	Copenhagen City Heart Study Programme	361	Male	0.41	0.36, 0.46	0.24	0.20, 0.29	0.16	0.12, 0.20
Yiannakouris et al. (54), 2001	Greece	Caucasian	Students	118	Mix	0.32	0.24, 0.41	0.12	0.06, 0.18	0.24	0.16, 0.31
de Silva et al. (55), 2001	Australia	Caucasian	Population-based study	335	Female	0.58	0.53, 0.63				
Overall		Caucasian		3,309 (Q223R)		0.45	0.42, 0.49	0.25	0.23, 0.28	0.18	0.16, 0.20
				2,498 (K109R)		$Q^* = 42.70$		$Q = 10.02$		$Q = 5.94$	
				2,886 (K656N)		$p < 0.0001$		$p = 0.124$		$p = 0.547$	
Matsuoka et al. (56), 1997	Japan	Asian	Population	115	Mix	0.85	0.79, 0.82	0.78	0.70, 0.85	0.12	0.06, 0.18
Endo et al. (57), 2000	Japan	Asian	School	553	Mix	0.85	0.82, 0.88				
Koh et al. (58), 2002	Korea	Asian	University	220	Male	0.85	0.80, 0.90	0.83	0.78, 0.88		
Overall		Asian		888 (Q223R)		0.85	0.84, 0.86	0.82	0.78, 0.86		
				335 (K109R)		$Q = 0.000$		$Q = 1.135$			
						$p = 1.000$		$p = 0.287$			
Thompson et al. (59), 1997	United States	Pima Indians	Population	20	Mix	0.25	0.06, 0.44	0.42	0.19, 0.64		
Stefan et al. (60), 2002	United States	Pima Indians	Population	452	Mix	0.32	0.28, 0.36				
Overall		Pima Indians		472		0.32	0.28, 0.36				
						$Q = 0.232$					
						$p = 0.630$					
Chung et al. (61), 1997	United States	Mix	Obesity center	194	Mix	0.34	0.27, 0.41	0.21	0.15, 0.27	0.15	0.10, 0.20
Mattevi et al. (62), 2002	United States	Brazilian European descent	Population	335	Mix	0.40	0.35, 0.46				
de Silva et al. (63), 1999	Australia	Nauruans	Population	232	Male	0.89	0.85, 0.93				

* *LEPR*, leptin receptor gene; CI, confidence interval; *Q*, Cochran *Q*-test for heterogeneity.

TABLE 3. Studies that included data on the C161T polymorphism of the *PPARG gene in healthy subjects**

Authors (reference no.), year	Country	Population	Source	No. of subjects	Gender	*161T* allele frequency	95% CI*
Valve et al. (64), 1999	Finland	Caucasian	Primary health care	141	Female	0.18	0.12, 0.25
Meirhaeghe et al. (65), 1998	France	Caucasian	WHO MONICA*	820	Mix	0.15	0.11, 0.18
Orio et al. (66), 2003	Italy	Caucasian	Volunteer	100	Female	0.07	0.02, 0.11
Wang et al. (67), 1999	Australia	Caucasian	Heart clinic	133	Mix	0.21	0.14, 0.28
Overall		Caucasian		1,194		0.16	0.12, 0.22
						$Q^* = 6.538$; $p = 0.088$	
Muller et al. (68), 2003	United States	Pima Indians	Population	330	Mix	0.41	0.36, 0.46
Ogawa et al. (69), 1999	Japan	Asian	Population	404	Female	0.15	0.11, 0.18

* *PPARG*, peroxisome proliferator-activated receptor-gamma gene; CI, confidence interval; WHO MONICA, World Health Organization Monitoring of Trends and Determinants in Cardiovascular Disease (study); *Q*, Cochran *Q*-test for heterogeneity.

(68, 70–73, 93), five in Asia (88–92), 14 in Europe (64, 74–86), and one in Oceania (87). The frequency of the *12Ala* allele was statistically different among ethnic groups, with Caucasians showing the highest frequency of this variant allele (table 4).

DISEASE

Obesity is a common condition in industrialized societies and is increasing rapidly; its etiology is complex and results from combined effects of genes, environment, lifestyle, and their interactions (94–99). Obesity is defined as an increase in body fat, while overweight is an increase in weight relative to a standard. In most studies, obesity is defined on the basis of anthropometric measures, mainly height, weight, and waist circumference. Such measurements give information on the degree and distribution of obesity (100). On this basis, subjects are defined as overweight if their body mass index (BMI; weight in kilograms divided by height in meters squared) is equal to or greater than 25 kg/m^2 and obese if their BMI is 30 kg/m^2 or more (i.e., weight exceeding 20 percent of the ideal weight), according to World Health Organization indications (101).

Several other ways to define obesity have been mentioned in the literature. Some studies have reported waist-to-hip ratio as a rough approximation of body fat distribution (102). In the elderly, sarcopenic obesity has been described, which is defined as an excess of fat with loss of lean body mass (103). Some studies have also described a form of ectopic deposition, which consists of an excess deposition of fat in muscle, liver, and pancreas resulting in insulin resistance and beta-cell dysfunction. The central adipose tissue responds to this unbalance by releasing large quantities of free fatty acids (104).

Body composition can also be estimated by using a variety of technical methods. Included are densitometry methods, such as hydrostatic weighing and plethysmography, bioelectrical impedance analysis, dual energy x-ray absorptiometry, and near-infrared interactance.

Obesity affects 10–25 percent of the European population and nearly one third of the US population, and the number continues to increase (105–107). Each year, obesity causes at least 300,000 excess deaths in the United States (http://www.obesity.org/). Risk factors for obesity include a poor diet consisting of low-nutrient but high-calorie foods, lack of physical activity, medical conditions such as rare hereditary diseases and a hormonal imbalance (e.g., hypothyroid disease), age, genetic factors, ethnicity, and gender (108, 109). Overweight and obesity increase steadily with age in both men and women and occur at higher rates in ethnic minority populations in the United States, such as African Americans and Hispanic Americans compared with White Americans, while the prevalence in Asian Americans is relatively low (110). Women and persons of low socioeconomic status within minority populations appear to be particularly affected by overweight and obesity (111). Cultural factors that influence dietary and exercise behaviors are reported to play a major role in the development of excess weight in minority groups. In these ethnic minorities, rates of several obesity-related diseases (diabetes, hypertension, cancer, and heart disease) are higher than those among Whites (112–115). Obesity is associated with medical conditions that can cause poor health and premature death, among which are arthritis, birth defects, various forms of cancer, cardiovascular disease, diabetes, hypertension, infertility, chronic venous insufficiency, deep vein thrombosis, end-stage renal disease, gout, impaired immune response and impaired respiratory function, liver disease, pancreatitis, sleep apnea, and stroke (116–118).

TABLE 4. Studies that included data on the Pro12Ala polymorphism of the *PPARG gene in healthy subjects**

Authors (reference no.), year	Country	Population	Source	No. of subjects	Gender	*12Ala* allele frequency	95% CI*
Beamer et al. (70), 1998	United States	Caucasian	Population, university weight center	686	Mix	0.11	0.09, 0.13
Memisoglu et al. (71), 2002	United States	Caucasian	Nurses' Health Study	953	Female	0.11	0.09, 0.13
Memisoglu et al. (72), 2003	United States	Caucasian	Nurses' Health Study	771	Female	0.13	0.11, 0.15
Memisoglu et al. (73), 2003	United States	Caucasian	Nurses' Health Study	2,141	Female	0.13	0.11, 0.14
Franks et al. (74), 2004	England	Caucasian	Medical Research Council	506	Mix	0.11	0.09, 0.14
Ringel et al. (75), 1999	Germany	Caucasian	Population	310	Mix	0.15	0.11, 0.19
Koch et al. (76), 2000	Germany	Caucasian	Relatives of diabetic subjects	108	Mix	0.18	0.10, 0.25
Evans et al. (77), 2000	Germany	Caucasian	Hospital, blood donors	392	Mix	0.15	0.12, 0.19
Evans et al. (78), 2001	Germany	Caucasian	Hospital	568	Mix	0.14	0.11, 0.17
Vigourux et al. (79), 1998	France	Caucasian	Unrelated persons	59	Mix	0.06	0.00, 0.12
Ek et al. (80), 1999	Denmark	Caucasian	Cohort	1,621	Male	0.15	0.13, 0.16
Frederiksen et al. (81), 2002	Denmark	Caucasian	Health survey	1,951	Mix	0.14	0.13, 0.16
Valve et al. (64), 1999	Finland	Caucasian	Weight reduction study	141	Female	0.14	0.08, 0.20
Deeb et al. (82), 1998	Finland	Caucasian	Population	1,306	Mix	0.15	0.13, 0.16
Niskanen et al. (83), 2003	Finland	Caucasian	Population	119	Mix	0.12	0.06, 0.18
Mancini et al. (84), 2003	Italy	Caucasian	Population	312	Mix	0.10	0.07, 0.13
Vaccaro et al. (85), 2000	Italy	Caucasian	Telephone company	375	Mix	0.16	0.13, 0.20
Gonzalez Sanchez et al. (86), 2002	Spain	Caucasian	Hospital	464	Mix	0.09	0.06, 0.12
Swarbrick et al. (87), 2001	Australia	Caucasian	Carotid disease study, population	663	Mix	0.14	0.11, 0.16
Overall		Caucasian		13,446		0.13	0.12, 0.14
						$Q^* = 36.20$; $p = 0.004$	
Mori et al. (88), 1998	Japan	Asian	Hospital	215	Male	0.03	0.01, 0.05
Hara et al. (89), 2000	Japan	Asian	Health center	541	Mix	0.04	0.02, 0.06
Yamamoto et al. (90), 2002	Japan	Asian	Hospital	595	Mix	0.03	0.01, 0.04
Kahara et al. (91), 2003	Japan	Asian		123	Male	0.02	0.00, 0.05
Lei et al. (92), 2000	Taiwan	Asian	Hospital	310	Mix	0.04	0.02, 0.06
Deeb et al. (82), 1998	Finland	Asian	Population	54	Mix	0.09	0.02, 0.17
Overall		Asian		1,838		0.04	0.03, 0.05
						$Q = 3.32$; $p = 0.506$	
Kao et al. (93), 2003	United States	African American	Arteriosclerosis Risk in Communities study	1,172	Mix	0.02	0.01, 0.03
Muller et al. (68), 2003	United States	Pima Indians	Population	330	Mix	0.09	0.06, 0.12

* *PPARG*, peroxisome proliferator-activated receptor-gamma gene; CI, confidence interval; *Q*, Cochran *Q*-test for heterogeneity.

TABLE 5. Description of the case-control studies included in the meta-analyses and of the polymorphisms tested in each study

Authors (reference no.)	Population	No. of cases	No. of controls	Obesity definition cutoff	Polymorphisms tested
Silver et al. (46)	Caucasian	281	107	BMI*	Q223R, K656N
Gotoda et al. (47)	Caucasian	190	132	BMI	Q223R, K109R, K656N
Mammès et al. (49)	Caucasian	277	289	BMI $\geq$27 kg/m^2	Q223R, K109R, K656N
Echwald et al. (53)	Caucasian	156	205	BMI	Q223R, K109R, K656N
Yiannakouris et al. (54)	Caucasian	29	89	BMI $>$25 kg/m^2	Q223R, K109R, K656N
Matsuoka et al. (56)	Asian	47	68	BMI $>$30 kg/m^2	Q223R, K109R, K656N
Endo et al. (57)	Asian	90	463	Obesity index†	Q223R
Thompson et al. (59)	Pima Indians	10	10	% body fat	Q223R, K109R
Chung et al. (61)	Mix	167	27	BMI	Q223R, K109R, K656N
Mattevi et al. (62)	Brazilian	183	152	BMI $\geq$25 kg/m^2	Q223R
Beamer et al. (70)	Caucasian	169	517	BMI	P12A
Ek et al. (80)	Caucasian	752	869	BMI $\geq$31 kg/m^2	P12A
Vaccaro et al. (85)	Caucasian	95	280	BMI $>$35 kg/m^2	P12A
Gonzalez Sanchez et al. (86)	Caucasian	145	317	BMI $\geq$30 kg/m^2	P12A
Swarbrick et al. (87)	Caucasian	292	371	BMI $\geq$30 kg/m^2	P12A
Mori et al. (88)	Asian	169	46	BMI $\geq$22 kg/m^2	P12A

* BMI, body mass index (weight (kg)/height (m^2)).
† Obesity index = (real weight – standard weight)/(standard weight $\times$ 100).

ASSOCIATIONS

LEP A19G

Only one case-control study, which was conducted in Finland (29) and included 141 cases and 65 controls, was available on the association between this polymorphism and obesity. The observed odds ratio was 1.04 (95 percent CI: 0.67, 1.61).

LEPR Q223R

Ten studies satisfied the selection criteria and were included in the meta-analysis (46, 47, 49, 53, 54, 56, 57, 59, 61, 62) (table 5). The overall odds ratio was 1.13 (95 percent CI: 0.98, 1.30) (figure 1A), with no evidence of statistical heterogeneity nor of publication bias (figure 2A).

LEPR K109R

Seven studies were considered for the meta-analysis (47, 49, 53, 54, 56, 59, 61) (table 5). The overall association between *LEPR* K109R and obesity was 1.05 (95 percent CI: 0.89, 1.23) (figure 1B). The Cochran *Q*-test showed homogeneity among studies; the funnel plot showed no evidence of publication bias (figure 2B).

LEPR K656N

Seven studies were included in this meta-analysis (46, 47, 49, 53, 54, 56, 61) (table 5). The overall odds ratio was 1.02 (95 percent CI: 0.86, 1.21) (figure 1C), with no evidence of statistical heterogeneity nor of publication bias (figure 2C).

PPARG C161T

Of seven articles reviewed, none included data for both lean and obese subjects but instead reported information on one or the other of the two categories. Therefore, no formal statistical analysis was performed on these data.

PPARG P12A

Six studies were considered in this meta-analysis (70, 80, 88, 85–87) (table 5). The overall association between *PPARG* P12A and obesity was 1.13 (95 percent CI: 0.98, 1.29) (figure 1D). The Cochran *Q*-test showed that the results of the different studies were distributed homogeneously, with no statistical evidence of publication bias from the funnel plots (figure 2D).

INTERACTIONS

Gene-environment interactions

Since leptin is involved in weight regulation, it is interesting to assess whether any interaction exists between leptin polymorphisms and diet or gender. Polymorphisms in the *LEPR* gene have been studied as possible modifying factors of the response to diet (28) or of survival in cancer patients according to their BMI (119), but the associations, if present, represented secondary subgroup analyses of the data.

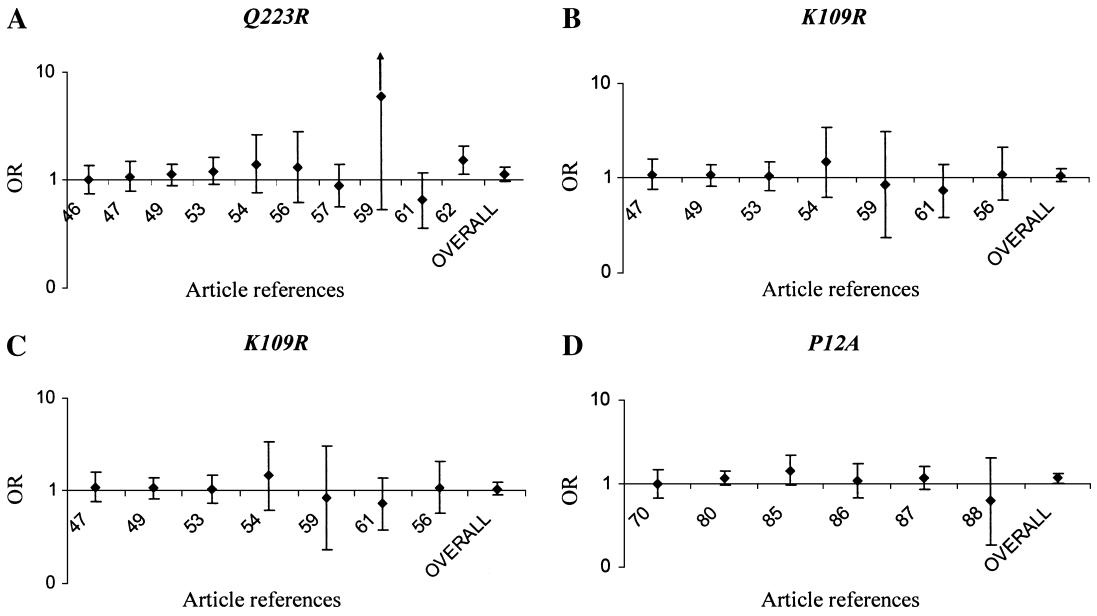

FIGURE 1. Results of a meta-analysis of the association between leptin polymorphisms and obesity. Data are presented as standard forest plots. Numbers on the *x*-axes, articles cited in the References section of the text. The pooled odds ratios (ORs) were obtained by using fixed- or random-effects models. Refer to the Associations section of the text for information on parts A–D.

A pooled analysis (120) and a meta-analysis (121) suggested an interaction between gender and the *R109R* genotype on BMI, although the reported effect was modest. A study in a Brazilian population indicated that the association between *LEPR* Q223R and BMI was stronger in nonsmokers than in the general population (62). The presence of a gene-diet interaction was studied in 592 nondiabetic subjects genotyped for the P12A variant of *PPARG* (122). Results showed that when the ratio of polyunsaturated to saturated fats in the diet was low, BMI was greater in *Ala* carriers than in *Pro* homozygous subjects. The opposite effect was seen when the ratio was high. An analysis of several genes involved in regulation of body weight suggested that the allelic variants of *LEP* and *PPARG* might affect diet-related obesity risk (123).

Gene-gene interactions

Interactions between polymorphisms involved in regulation of leptin have been reported. An interaction between polymorphisms in the *LEP* and *LEPR* genes on the risk of non-Hodgkin's lymphoma has been suggested (124) and on insulin levels in the population of Nauruans (63). An interaction between the *109R* and *223R* variants on blood pressure levels was suggested (52), as was one between the K109R and K656N polymorphisms on BMI (120, 121).

DISCUSSION

Systematic screenings of the genome have allowed several polymorphisms in the genes involved in leptin regulation to be identified. In this review, we have reported data on the association between obesity and a polymorphism in the *LEP* gene (A19G), three polymorphisms in the *LEPR* gene (Q223R, K109R, and K656N), and two polymorphisms in the *PPARG* gene (C161T and P12A). A meta-analysis performed on the data suggests no evidence of an association between the genes involved in leptin regulation and obesity. The analysis stratified according to ethnicity did not show any variation across populations, in agreement with published literature on this topic (125). Functional data on these genetic variants are very limited and are consistent with a lack of association between these polymorphisms and obesity. The results of our analysis are consistent with those observed in another meta-analysis of 20 studies on 3,263 subjects of different ethnic groups (62 percent of whom were family members of one or more subjects in the data set), which reported no significant effect of the polymorphisms of *LEPR* on obesity (121). In a meta-analysis by Masud and Ye (126), the P12A polymorphism was associated with BMI in markedly obese persons. It has been suggested that P12A is a functional polymorphism and that substitution of alanine for proline leads to a decrease in *PPARG* activity, thus reducing its ability to regulate expression of the gene (82). The P12A polymorphism has been associated with diabetes, although the reported effect is modest (126). Our data do not indicate an association between obesity and the P12A polymorphism.

One strength of the present meta-analysis is that, to our knowledge, this is the first such study performed exclusively on unrelated, healthy subjects. A possible limitation is that we could evaluate only the allele frequency of the different polymorphisms, since very few studies reported

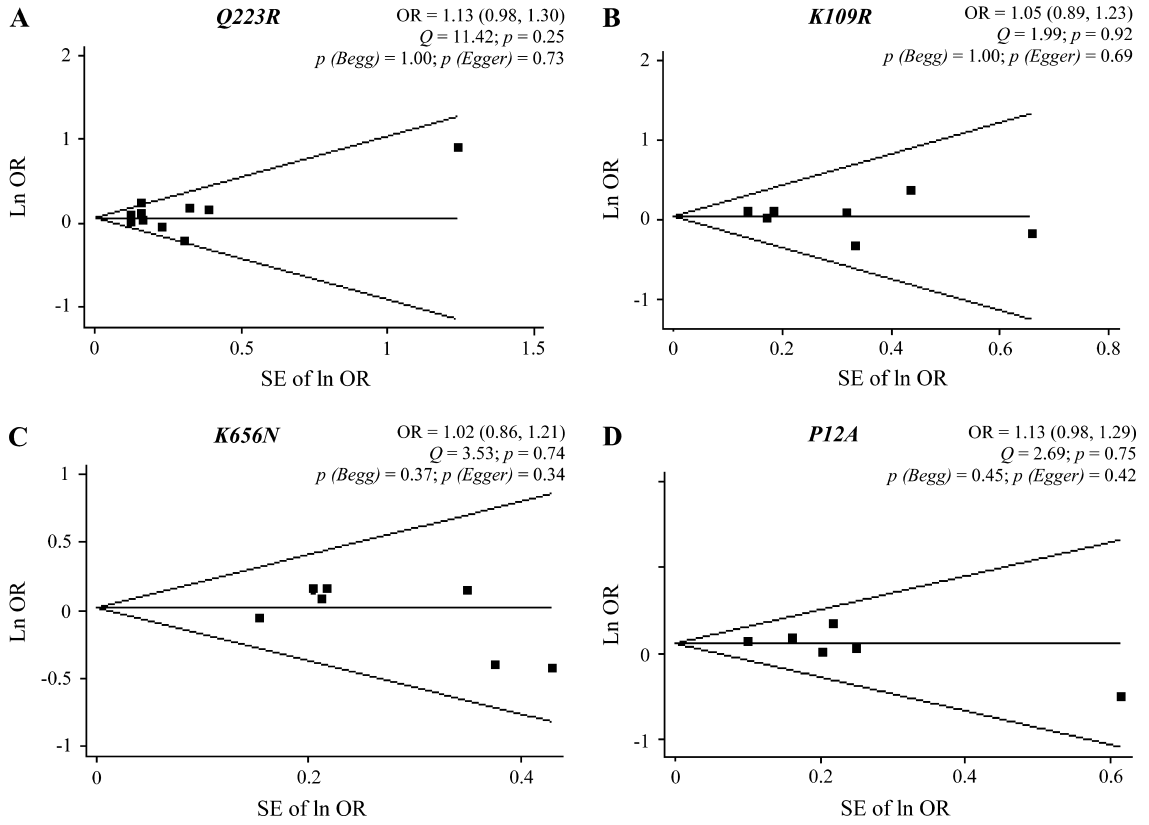

FIGURE 2. Funnel plots of the natural logarithm of the odds ratio (LnOR) vs. the standard error (SE) of the natural logarithm of the odds ratio for studies on the association between leptin polymorphisms and obesity. Numbers in parentheses, 95% confidence interval. *Q*, Cochran *Q*-test for heterogeneity; *p* (Begg), *p* value for Begg's test for publication bias; *p* (Egger), *p* value for Egger's test for publication bias. Refer to the Associations section of the text for information on parts A–D.

the genotype frequencies. Another limitation is that the number of case-control studies conducted on healthy subjects is still small; several studies reported data on only lean or on only obese persons, but these studies could not be included. The cutoff for obesity, when specified, differed among studies; however, we had to use the definition given by each author, since this was a meta-analysis of published data.

The reported lack of association between leptin polymorphisms and obesity could be due to the complex pathogenesis of obesity, which involves a large number of both genetic and environmental factors. In addition, it should be taken into account that the results were based on studies that used BMI as a marker for the obesity phenotype, while several other methods of defining this condition are available. Therefore, studies including different measures of obesity could be useful in this field.

LABORATORY TESTS

The detailed methods used for determining the different polymorphisms are described in each article. All studies included in the present analysis used genomic DNA extracted from blood. Thirty-one articles reported the use of polymerase chain reaction followed by restriction fragment length polymorphism, 10 used polymerase chain reaction followed by single strand conformation polymorphism; three studies used Southern blot, four studies sequencing, and one hybridization techniques in addition to polymerase chain reaction.

POPULATION SCREENING

Attempts to relate DNA sequence variation in specific genes to obesity phenotypes continue to grow. The obesity gene map shows putative loci on all chromosomes except Y. Overall, more than 430 genes, markers, and chromosomal regions have been associated or linked with human obesity phenotypes (127). However, neither the biologic nor the epidemiologic data on these genes are complete enough to make them candidates for screening in obese people. Before planning a screening program, the association between the gene and the disease should be established with some degree of certainty, and efficacious preventive intervention targeting at-risk subjects should be available. Considering these premises, there is insufficient evidence at present to

justify population testing for the *LEP*, *LEPR*, or *PPARG* polymorphisms in an obesity screening program in the general population.

CONCLUSION AND RESEARCH PRIORITIES

Obesity is a major public health concern given the association of this condition with several chronic diseases. Identification of genetic variants that increase a person's susceptibility to the common forms of obesity is a critical problem. Several recent studies have made an attempt in this direction (128). The main summary of the literature indicates no association between the genes involved in leptin regulation and obesity. It is also evident from this review that the pathogenesis of obesity is complex and that the interaction between genetic and environmental factors is a crucial event.

Further areas of research include both gene-gene and gene-environment interaction as well as individual genetic and metabolic profiles using the developments occurring in genomics and proteomics (129). Larger studies on both obese and lean subjects are needed, with testing of multiple genes and detailed epidemiologic data on the dietary habits of different ethnic groups, including a better definition of the obesity phenotype.

INTERNET SITES

Online Mendelian Inheritance in Man (OMIM). Center for Medical Genetics, Johns Hopkins University (Baltimore, Maryland) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Maryland), 1999: http://www.ncbi.nlm.nih.gov/omim/

American Obesity Association (AOA): http://www.obesity.org/

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